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Variability of *Fasciola hepatica* infection in *Lymnaea ovata* in relation to snail population and snail age

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Abstract Bimiracidial infections of *Lymnaea ovata* with *Fasciola hepatica* were performed under laboratory conditions to determine the susceptibility of snails from six French populations to trematode infection. In five populations of *L. ovata* the prevalence of infection in the 1-mm groups ranged between 2.7% and 43.7% at day 35 postexposure; it decreased in the 2-mm snails and was zero in larger groups. In the snails from Thenay (periodically polluted brook) the prevalence of *F. hepatica* infection decreased from the 1-mm group to the 8-mm group (from 23.9% to 1.0%) and was zero in the 10-mm *L. ovata*. The total number of cercariae shed per snail was 18.3 in the 1-mm group, increasing to 117 in the 8-mm group. The latter findings could be interpreted as a consequence of periodic pollution in the brook of Thenay; pollution might disrupt the defense system of *L. ovata* and facilitate the subsequent larval development of *F. hepatica*.

Introduction

Some freshwater snails can act as intermediate hosts in the life cycle of *Fasciola hepatica*. The one most cited in western Europe is *Lymnaea truncatula* (Boray 1969). However, this species is not the only intermediate host, as fascioliasis in ruminants may be detected in farms that lack *L. truncatula* (Abrous et al. 1998). The European populations of other *Lymnaea* species are also susceptible to artificial infection, and full development of *F. hepatica* may occur in very young specimens of *L. glabra*, *L. ovata*, *L. palustris*, *L. peregra*, *L. stagnalis* (Kendall 1950; Boray 1978), and *Myxas glutinosa* (Vareille-Morel et al. 1994).

The current status of our knowledge on the intermediate hosts of *F. hepatica* has failed to explain the high prevalence of infection in cattle living in French departments with coastal marshes, such as Charente Maritime and Manche (Mage et al. 1989; Cadel et al. 1996). Populations of *L. truncatula* were rare and comprised fewer than 50 snails. In contrast, *L. ovata* proliferated in irrigation canals. The presence of the latter species in this environment raises the question of whether natural infection of this snail with *F. hepatica* would occur, as the mortality of snails during the course of infection was high and the number of cercariae released by each snail was often lower than 20 (Busson et al. 1982). In view of these findings, the following two questions arose: what was the susceptibility of *L. ovata* to *F. hepatica* infection when the geographic origin of snail populations and their types of habitat differed? What were the characteristics of *F. hepatica* infection in *L. ovata* under laboratory conditions? To answer the first question we performed an experiment by subjecting 1- to 4-mm *L. ovata* from six French populations to bimiracidial exposures and investigating the success of parasitic infection at day 35 postexposure (p.e.). To answer the second question we studied the cercarial shedding of *F. hepatica* in a single population of *L. ovata*, as infected snails were found in the 3-mm and larger groups of this colony during the first experiment.

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Materials and methods

The first population of *Lymnaea ovata* (Fig. 1) was living in an irrigation canal running through the coastal marsh of Beauvais,

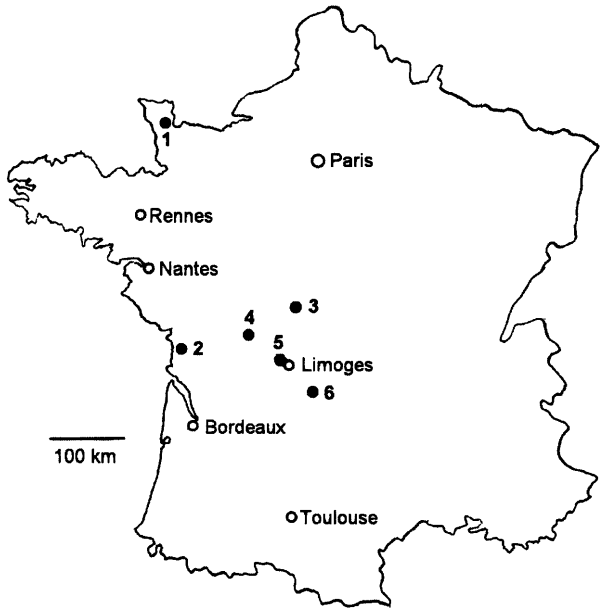

Fig. 1 The location of the six populations of *Lymnaea ovata* in France (*1* Gourbesville, Manche; *2* Fouras, Charente Maritime; *3* Thenay, Indre; *4* Lathus Saint Rémy, Vienne; *5* Rilhac Rancon, Haute Vienne; *6* Juillac, Corrèze)

commune of Gourbesville, department of Manche. The second population originated from another irrigation canal in a coastal marsh near the farm of Nauleries, commune of Fouras, department of Charente Maritime. The third, fourth, and fifth populations were living in small brooks in the departments of Indre (Thenay), Vienne (farm of La Pennetrie, commune of Lathus Saint Remy), and Haute Vienne (La Mazelle, commune of Rilhac Rancon), respectively. Finally, the sixth population inhabited a pond at the farm of Les Plaines, commune of Juillac, department of Correze. Pollution with household refuse and the use of a herbicide (Roundup Bioforce, Monsanto, Belgium; 2 spreadings/year consisting of 80 l of water each, with 12 g of herbicide/l) was periodically noted in the brook of Thenay, whereas the other habitats had no known pollution at the time of experimentation. Adult *L. ovata* were collected at these sites and were maintained under laboratory conditions in aquaria until they had laid eggs. Progeny snails measuring 1–10 mm in height were subsequently used for miracidial exposure. Eggs of *Fasciola hepatica* were collected from the gallbladders of cattle with heavy infections. The cattle originated from two farms located in the west of Haute Vienne department. Eggs were incubated for 20 days at 20 °C in complete darkness prior to miracidial hatching.

In the first experiment, 4 groups of 100 snails each were constituted for each population. Snails from the first group of each population were 1 mm high (corresponding to 1–3 days of life), whereas those from the second, third, and fourth groups were 2 mm (3–7 days), 3 mm (8–13 days), and 4 mm (14–18 days) high, respectively. The snails from the 24 groups were individually exposed to 2 miracidia of *F. hepatica* for 4 h. They were subsequently raised until day 35 p.e. in open boxes ($100 \times 60 \times 15$ cm) at a density of 50 snails/box (Abrous et al. 1999). The breeding boxes were placed in an air-conditioned room under the following conditions: a constant temperature of 20 °C, a diurnal photophase, and a light intensity of 3,000–4,000 lux over the boxes. At day 35 p.e. the survivors of each group were dissected under a stereomicroscope for determination as to whether the snails were infected.

The second experiment was performed only with the *L. ovata* from Thenay, as infected snails were found in the 3- and 4-mm groups in the first experiment. In all, 6 control groups of 100 snails each were constituted using *L. ovata* measuring 1, 2, 4, 6, 8, or 10 mm, respectively. The same protocol was used for the 6 groups of snails exposed to miracidia of *F. hepatica* (2 miracidia/snail for 4 h). The snails from the 12 groups were subsequently raised until day 36 in open boxes at 20 °C. At day 35 p.e., survivors in the control and exposed groups were individually isolated in 35-mm-diameter petri dishes containing 2 or 3 ml of water and a piece of lettuce. The recipients were placed in the same air-conditioned room as the boxes. Every day a metacercarial count was performed, and the water in the dish was changed until snail death.

The first two parameters determined were the survival rate at day 35 p.e. in each group and the prevalence of snail infection. The latter frequency took into account snails harboring larval forms of *F. hepatica* (first experiment) or snails shedding cercariae (second experiment) and was calculated in each snail group as the ratio between the number of infected snails and that of surviving snails at day 35 p.e. Four other parameters were also studied in the second experiment: the increase in the shell height of controls and cercaria-shedding snails over the first 60 days p.e., the interval between exposure and the first cercarial shedding, the duration of the shedding period, and the total number of metacercariae produced by each infected snail. Mean values and SD were determined for each of the latter four parameters. Calculations of confidence intervals at a probability of 0.95 (CI, $P = 0.95$), the Pearson correlation test, and one- or two-way analysis of variance were used to establish levels of significance.

Results

Susceptibility of *Lymnaea ovata* populations to *Fasciola hepatica* infection: first experiment

Table 1 gives the values recorded for the 2 parameters evaluated in the 24 groups. In five populations the number of *L. ovata* surviving at day 35 p.e. significantly increased with increasing shell height at exposure (Gourbesville: $r = 0.99$, $P < 0.01$; Fouras: $r = 0.99$, $P < 0.05$; Lathus Saint Rémy: $r = 0.99$, $P < 0.01$; Rilhac Rancon: $r = 0.99$, $P < 0.01$; Juillac: $r = 0.98$, $P < 0.05$). The snail population ($P < 0.001$) and the shell height at exposure ($P < 0.001$) had a significant influence on the survival rates. Second, the prevalence of *F. hepatica* infection at day 35 p.e. varied in relation to the snail population, i.e., from 2.7% in the 1-mm group from Lathus Saint Rémy to 43.7% in that from Gourbesville. However, in each population the percentages decreased significantly with increasing shell height at exposure. The snail population ($P < 0.01$) and the snail height at exposure ($P < 0.01$) had an influence on the prevalence of *F. hepatica* infection. Noteworthy is the prevalence of infection found in the 3- and 4-mm groups from Thenay (3.1% and 1.2%, respectively) as compared with the absence of infected snails observed in the corresponding groups among the other five populations of *L. ovata*.

Characteristics of infection in the snails from Thenay

Table 2 gives the values recorded for the two parameters in these groups. Positive correlations between the increase in the survival rate at day 35 p.e. and the shell height at exposure were found in the controls ($r = 0.88$, $P < 0.02$) as well as in the exposed snails ($r = 0.96$,

Table 1 The survival rate of *Lymnaea ovata* at day 35 p.e. and the prevalence of *Fasciola hepatica* infection in the 24 groups of exposed snails (first experiment)

Snail population and shell height at exposure	Number of snails[a]		Survival rate (%) at day 35 p.e.[b]	Prevalence (%) of *Fasciola* infection[b]
	At day 35 p.e.	With larval forms		
Gourbesville				
1 mm	48	21	48.0 ± 9.7	43.7 ± 14.0
2 mm	59	7	59.0 ± 9.6	11.8 ± 8.2
3 mm	71	0	71.0 ± 8.8	0
4 mm	78	0	78.0 ± 8.8	0
Fouras				
1 mm	37	11	37.0 ± 9.4	29.7 ± 15.4
2 mm	45	7	45.0 ± 9.7	15.5 ± 12.2
3 mm	51	0	51.0 ± 9.7	0
4 mm	63	0	63.0 ± 9.4	0
Thenay				
1 mm	55	11	55.0 ± 9.7	20.0 ± 10.5
2 mm	52	7	52.0 ± 9.7	9.6 ± 8.0
3 mm	63	2	63.0 ± 9.4	3.1 ± 4.2
4 mm	77	1	77.0 ± 8.2	1.2 ± 2.4
Lathus Saint Rémy				
1 mm	37	1	37.0 ± 9.4	2.7 ± 5.2
2 mm	48	0	48.0 ± 9.7	0
3 mm	59	0	59.0 ± 9.6	0
4 mm	77	0	77.0 ± 8.8	0
Rilhac Rancon				
1 mm	65	6	65.0 ± 9.3	9.2 ± 7.0
2 mm	71	2	71.0 ± 8.8	2.8 ± 3.8
3 mm	83	0	83.0 ± 7.2	0
4 mm	91	0	91.0 ± 5.0	0
Juillac				
1 mm	54	7	54.0 ± 9.7	12.9 ± 8.2
2 mm	71	2	71.0 ± 8.8	2.8 ± 3.8
3 mm	88	0	88.0 ± 6.3	0
4 mm	94	0	94.0 ± 5.1	0

[a] 100 snails/group at miracidial exposure
[b] CI 95 (confidence interval at a probability of 0.95)

Table 2 The survival rate of *L. ovata* at day 35 p.e. and the prevalence of *F. hepatica* infection in the groups from Thenay (second experiment)

Shell height of snails at exposure	Number of snails[a]		Survival rate (%) at day 35 p.e.[b]	Prevalence (%) of *Fasciola* infection[b]
	At day 35 p.e.	With shedding		
1-mm groups				
Controls	92	0	92 ± 5.3	0
Exposed snails	46	11	46 ± 9.7	23.9 ± 5.8
2-mm groups				
Controls	96	0	96 ± 3.8	0
Exposed snails	62	8	62 ± 9.5	12.9 ± 4.6
4-mm groups				
Controls	98	0	98 ± 2.7	0
Exposed snails	76	4	76 ± 8.3	5.2 ± 3.0
6-mm groups				
Controls	99	0	99 ± 1.9	0
Exposed snails	81	2	81 ± 7.6	2.4 ± 1.9
8-mm groups				
Controls	100	0	100	0
Exposed snails	91	1	91 ± 5.6	1.1 ± 1.9
10-mm groups				
Controls	100	0	100	0
Exposed snails	96	0	96 ± 3.8	0

[a] 100 snails/group at miracidial exposure
[b] CI 95 (confidence interval at a probability of 0.95)

$P < 0.01$). The prevalence of *F. hepatica* infection decreased significantly from the 1-mm group to the 8-mm snails ($r = -0.94$, $P < 0.02$). Significant differences in survival rates as well as in the prevalence of infection in exposed snails can be noted in Table 2.

The increase in shell height over the first 60 days of experimentation (Fig. 2) became greater when the shell height at exposure increased. Positive correlations between these two parameters were noted in controls ($r = 0.99$, $P < 0.01$) as well as in cercaria-shedding snails ($r = 0.99$, $P < 0.01$). In the 1-mm group the growth of cercaria-shedding snails was significantly lower ($F = 15.45$, $P < 0.001$) than that recorded for controls. Similar findings were also noted in the 2-mm ($F = 8.85$, $P < 0.01$), 4-mm ($F = 5.53$, $P < 0.05$), and 6-mm ($F = 15.75$, $P < 0.001$) groups.

The interval between exposure and the first cercarial shedding (Fig. 3) ranged between 44.5 and 51.2 days. However, no significant difference between the mean values recorded for this parameter in the different groups was noted. The duration of the shedding period (Fig. 3) was shorter, with mean values ranging between 10.5 and 15.1 days. No significant variation between these groups was found.

The number of metacercariae detected per infected snail (Fig. 4) increased significantly ($r = 0.94$, $P < 0.02$) with increasing shell height at exposure from 18.3 in the 1-mm group to 117 in the 8-mm group. Snails in the 6-mm group produced a significantly higher number of parasites than did those in the other groups ($F = 3.47$, $P < 0.01$).

Discussion

Our results show how the susceptibility of *Lymnaea ovata* to *Fasciola hepatica* infection varied with the population studied. The prevalence of infection recorded in *L. ovata* fell within the range of values previously given by some authors for this lymnaeid species when juvenile snails were experimentally infected with

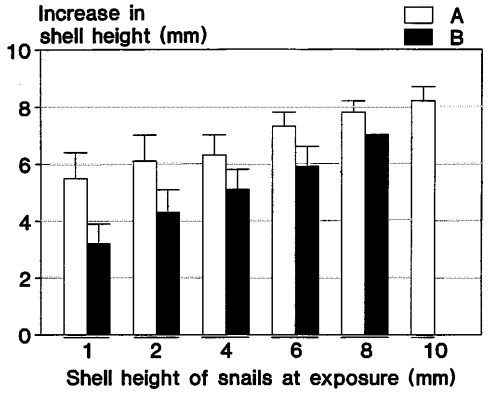

Fig. 2 Mean values and SD recorded for the increase in shell height in the groups from Thenay over the first 60 days of experimentation (*A* Controls, *B* cercaria-shedding *Lymnaea ovata*)

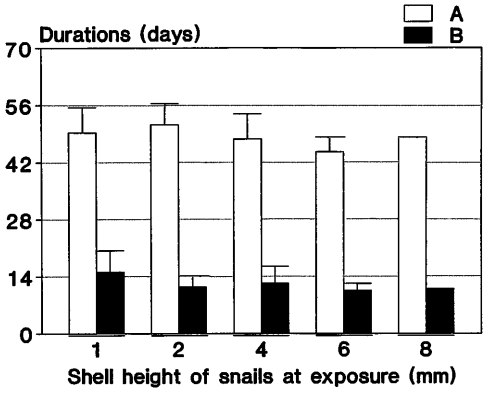

Fig. 3 Mean values and SD recorded for 2 parameters in the snail groups from Thenay: the interval between exposure and the first cercarial shedding (*A*) and the duration of the shedding period (*B*)

F. hepatica. In two populations from central France, Busson et al. (1982) reported that 0.4% and 20.4% of *L. ovata*, respectively, became infected when each newborn snail was exposed to three miracidia of *F. hepatica*. Infection rates were clearly lower (0.9–6.8%) in 0.5- and 1-mm *L. ovata* originating from French coastal marshes and individually exposed to five miracidia (Mage et al. 1989). These quantitative variations in the prevalence of *F. hepatica* infection among snail populations can mainly be explained by the work by Rondelaud (1993) in *L. truncatula*; according to this author the prevalence of infection in snails kept under laboratory conditions was low if the possibilities of a natural encounter between snails and trematodes were scarce. Under these conditions it is reasonable to assume that populations of *L. ovata* originating from Juillac, Lathus Saint Rémy, and Rilhac Rancon would not be adapted to *Fasciola* infection, whereas snails living in coastal marshes (Fouras and Gourbesville) would be more likely to have contact with the parasite in their natural habitat. If this hypothesis were accepted as valid, it would explain the heavy infection of cattle by *F. hepatica* in these coastal areas (e. g., Mage et al. 1989; Cadel et al. 1996), since *L. ovata* was abundant in these marshes and,

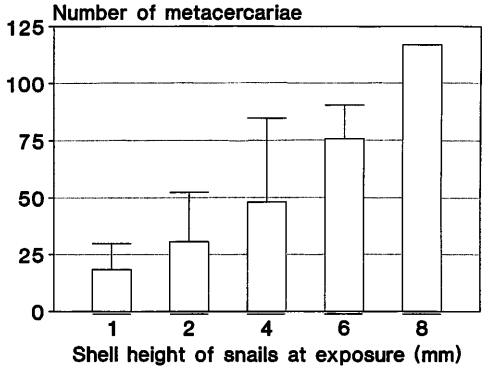

Fig. 4 Mean values and SD recorded for the number of metacercariae detected in the snail groups from Thenay

consequently, could sustain the larval development of this trematode when the populations of *L. truncatula* were rare or absent.

Another explanation may be given for the results obtained in the snails from Thenay. If the prevalence of *F. hepatica* infection decreased from 23.9% to 1% in bimiracidial exposures of 1- to 8-mm snails, respectively, it would also be interesting to consider the percentages given by Sindou et al. (1990, 1991) in the same population of *L. ovata*: 85.8% and 88.2% at day 45 p.e. in the single-miracidium infection of 1- and 2-mm snails, respectively. The aforementioned hypothesis did not stand up because (1) the brook is completely enclosed in the village of Thenay, (2) no cattle or sheep have been reared for over 30 years in this area, and (3) the presence of rabbits and rodents has rarely been noted. Thus, it appears that another explanation may be proposed to explain the high prevalence of *F. hepatica* infection reported by Sindou et al. (1990, 1991) in this population after its experimental exposure to miracidia. The most valid assumption would be to relate these findings to the periodic pollution of this brook with household refuse and herbicide spreading. The presence of these unusual factors could induce the development of tissue lesions in snails, as has been reported by Moukrim et al. (1988) in the same population of *L. ovata* following its exposure to a sublethal dose of trichlorfon, and could disrupt the defense system of the snail, which would facilitate the larval development of *F. hepatica*. One argument in support of this hypothesis would be that cercarial shedding was noted in the 1- to 8-mm snails from this population, whereas infected snails were found only in the 1- and 2-mm groups among the other colonies of *L. ovata*.

In the scope of the second experiment the mean number of metacercariae detected was 18.3 in the 1-mm group. This figure agreed with the numbers given by Busson et al. (1982) in *L. ovata* (mean 18.4 cercariae) and by Dreyfuss et al. (1997) in *L. peregra* infected at hatching and at 1-mm size (22.2 and 36.5 cercariae/snail). The increase in the number of metacercariae noted in upper-height *L. ovata* (to 117 in 8-mm snails) can be related to the shell growth occurring during the experiment, and these figures are entirely consistent with the values given by some authors (Kendall 1949; Kendall and Ollerenshaw 1963, among others) for the number of metacercariae recorded in *L. truncatula* experimentally infected with *F. hepatica*. Despite the low numbers, the cercarial production of *F. hepatica* was sufficient to ensure the infection of cattle present in coastal marshes, at least if one accepted the hypothesis of juvenile *L. ovata* intervening as intermediate hosts of *F. hepatica* in this environment.

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